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EVALUATION OF RADICLE EMERGENCE TEST TO PREDICT SEED VIGOUR AND FIELD EMERGENCE IN OKRA (*ABELMOSCHUS ESCULENTUS* L.) SEEDS

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ABSTRACT

An experiment was conducted to evaluate and correlate the radicle emergence test with other vigor parameters to predict the vigor and field emergence potential in ten seed lots of okra with varying vigor. The results showed that the significant differences were observed between seed lots in terms of physiological parameters viz., speed of germination, germination percentage (GER), root length, shoot length, dry matter production, vigor index, mean just germination time (MJGT), mean germination time (MGT), radicle emergence (RE) and field emergence (FE) and biochemical parameters viz., electrical conductivity of seed leachate (EC) and dehydrogenase activity (DA). The radicle emergence test with 2mm radicle length had highly significant negative correlation with MGT(-0.829**) followed by EC (-0.812**) and MJGT (-0.807**), and it had significant positive correlation with field emergence (0.904**) followed by germination (0.892**) and dehydrogenase activity (0.757*). Relationship between seed vigor parameters and radicle emergence per cent with 1mm and 2mm radicle length was also evaluated. The highest R^2 values were observed in 2mm radicle emergence per cent (R^2 Values: MJGT = 0.8839, MGT = 0.9223, EC = 0.8924, DA = 0.6335, GER = 0.9542 and FE = 0.9673) compared with 1mm length of radicle emergence per cent (R^2 Values: MJGT = 0.8812, MGT = 0.9156, EC = 0.8330, DA = 0.6204, GER = 0.9525 and FE = 0.9504) for most of the seed vigour parameters in ten different seed lots. The study concluded that counting 2mm radicle emergence at 24 hours could be used for a quick evaluation to assess the seed vigour in terms of field emergence in okra seed lots.

KEYWORDS: Radicle Emergence, Okra, Germination, Vigour & Field Emergence

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Original Article

INTRODUCTION

A seed is considered as the prime input in agriculture. Quality of the seed is most important to produce vigorous and healthy plants in the field. A key component of the performance of crop seeds in the field largely dependent on the seed vigor. Seed vigor, an important factor governing the seed quality which reflects on potential seed germination, seedling growth, seed longevity and tolerance to adversity (Sun *et al.*, 2007).

International Seed Testing Association defined that 'Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments'

(ISTA, 2014). Seed vigor is qualitative in concept than a quantifiable seed quality character, but it is often evaluated through various vigor tests where the vigor status is often indicated through comparative values. Vanderlip *et al.*(1973) observed that seed lots differed widely between each variable measured on standard germination and other vigor tests. A vigor test should provide a reproducible result which accurately describes the potential for rapid, uniform emergence under field conditions and describes the ranking of a seed lot. A practical seed vigor test should give a good indication of the field performance potential of the seed lot and the test results should be reproducible (Hampton and TeKrony, 1995).

Evaluation of seed vigor is important to predict the planting value of seed lot. The use of vigor tests, such as the determination of their physiological quality by seed-producing industries has been increasing (Marcos Filho, 2015). However, the vigor tests hope to select the seed lots with good storage capacity. The seed lots with the same germination per cent varying vigor in their performance either in storage and field (Carvalho and Nakagawa, 2000). Deterioration of seed lots is inevitable, which results in loss of seed vigor and viability (McDonald, 1999). Reduced vigor and viability of seeds may affect field performance and productivity of the subsequent crops (TeKrony and Egli, 1991). Therefore, the use of high-quality seed is mandatory, which necessitates the evaluation of its quality before introducing into the market. Seed vigor tests should be inexpensive, rapid, simple, objective, reproducible, and should have a high correlation with field performance (Copeland and McDonald, 2001).

The standard germination test is considered as the universal test for seed quality to evaluate the maximum potential of a particular seed lot only under an ideal set of environmental conditions (ISTA, 1987). A standard germination test is a time consuming and doesn't always show seed lot of potential performance, especially if field conditions are not optimal (Hampton and TeKrony, 1995).

Seed lots that do not differ in germination may differ in deterioration level and may differ substantially in field performance, thereby a vigor test is considered a powerful when it classified the seed lots into more groups or levels (Kolasinska*et al.*, 2000). Radicle emergence test is considered as a quick test to predict varying vigor level and field performance of seed lots than the standard germination test in several crops. RE test is important physiological traits, the electrical conductivity of seed leachate and dehydrogenase enzyme activity also important biochemical traits for assessing the seed quality. Radicle emergence is defined as the appearance of a radicle after breaking through the seed coat. The radicle emergence test has been accepted as a valid seed quality test by the International Seed Testing Association (ISTA) in the Annual Meeting held at Zurich in June 2011 for *Zea mays*(Matthews and Powell, 2011).

Development of radicle emergence as a vigor test will encourage the focused application of automated counting at an appropriate time without the need to determine the complete time course of germination. The radicle emergence test provides small laboratories with the opportunity to gain early information to predict the normal seedlings. Farmers easily practiced for radicle emergence test and do not require sophisticated equipment or highly skilled personnel and it could be used to shorten the decision period in the seed industry management(Mavi*et al*., 2016).

Okra (*Abelmoschusesculentus* L.) is one of the important vegetable crop of the tropical countries and most popular in India, Nigeria, Pakistan, Cameroon, Iraq and Ghana. It belongs to the family Malvaceae. India is the largest producer of okra with an area of 514 ha, production of 6126 MT, the productivity of 12 t/ha followed by Nigeria. Okra is rich in vitamin A and folic acid, besides phosphorus, magnesium, and potassium. It contains carbohydrate (5.4 %), protein (4 %) and total fat (0.5 %)(Indiastat, 2018).

According to Indian minimum seed certification standards (IMSCS) okra seedlings evaluated on the 21st day for its germination percentage, which is almost like a month. During this evaluation period, the seeds will be kept ideal. Once the seeds meet IMSCS it can be sold in the market. Since the seed is a living entity keeping it for one month not only reduced the germination percentage but also the vigor. We need to advance technology which would give a precise result in short period.

With this backdrops, the present study was undertaken with the aim to evaluate the radicle emergence test to predict seed vigor and field emergence in okra seed lots.

MATERIALS AND METHODS

The present experiment was conducted to evaluate and correlate the radicle emergence test with other vigor parameters in ten different seed lots (L_1 to L_{10}) of okra. Genetically pureten seed lots of okra var. Raja obtained from Dharani seeds, Udumalai pet formed the base material for this study. The laboratory and green-house experiments studies were carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2018-2019.

Standard Germination Test

The germination test was conducted with 100 seeds in four replications for each lot in the sand medium. The test conditions of 25 ± 2º C temperature and 95 ± 2 % RH were maintained in the germination room. At 21st day end of the germination test period, the number of normal seedlings was counted and the mean was expressed as germination percent (ISTA, 2016).

The seeds showing plumule emergence in each lot, replication wise were counted daily from the third day after sowing till the end of a germination test. From the number of seeds germinated on each day, the speed of germination was calculated as per the method suggested by Maguire, (1962) and the results were expressed in a number.

At the time of germination count, ten normal seedlings were selected at random from each lot replication wise and the root length was measured from the collar region to the tip of the primary root. The mean values were calculated and expressed in centimeter. The seedlings used to measure root length were also used for measuring shoot length. The shoot length was measured from the collar region to the tip of the primary leaves and the mean values were expressed in centimeter.

For dry matter production, the seedlings selected for root and shoot length measurements were put inside a paper cover, first shade dried for 24 h and then dried in a hot air oven maintained at 80 ± 2°C for 24 h. After cooled in silica gel desiccator for 30 min, the dry weight of seedlings was weighed using an electronic balance and the mean values were expressed in g / 10 seedling.

Vigour index values were computed using the following formula suggested by Abdul-Baki and Anderson (1973). The mean values were expressed as whole numbers.

Vigour index = Germination (%) x seedling length (cm)

Radicle Emergence (RE) Test

Radicle emergence test was conducted through Top of the paper method. Eight replicates of 25 seeds in each lot were placed on germination paper moistened with distilled water in petri - dish. The Petri - dishes were kept in a germination room maintained at 25±2 °C and relative humidity of 95 ± 2 %. The number of seeds that had produced the radicle of 1mm and 2 mm long was recorded from the initiation of radicle emergence at two hours interval up to 24 hours for each replication(ISTA, 2012).

From the daily count data, the percentage radicle emergence (1mm and 2mm), Mean Just Germination Time (MJGT) and Mean Germination Time (MGT) were calculated using the following formula.

$$\text{Radicle emergence with 1mm length(\%)} = \frac{\text{No. of seeds with 1mm radicle length}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Radicle emergence with 2mm length(\%)} = \frac{\text{No. of seeds with 2mm radicle length}}{\text{Total no. of seeds sown}} \times 100$$

The first appearance of the radicle, which is termed as Mean Just Germination Time and the Mean Germination Time, which is the mean lag period, to radicle emergence was calculated using the following formula proposed by Ellis and Roberts (1980) and expressed in hours.

MJGT = $\Sigma nD / \Sigma n$

{Where, n= number of seeds germinated (first appearance of the radicle) at time D, D= hours from the beginning of the germination test, Σn= final number of radicle emergence}

MGT = $\Sigma nD / \Sigma n$

{Where, n= number of seeds germinated (with 2 mm radicle emergence) at time D, D= hours from the beginning of the germination test, Σn= final number of radicle emergence}

Electrical Conductivity (EC) of Seed Leachate Test

Four replicates of twenty-five seeds in each seed lot were prewashed with distilled water to remove the adhering chemicals and then soaked in 50 ml of distilled water for 12 hours at room temperature. After soaking, the seed steep water was decanted to obtain the seed leachate. The electrical conductivity of the seed leachate was measured in a digital conductivity meter with a cell constant of one and expressed as dSm^{-1}(Presley, 1958).

Dehydrogenase Activity (DA) Test

Four replicates of twenty-five seeds in each seed lot were pre-conditioned by soaking in water for 12 hours at room temperature. Out of this, 10 seeds were taken at random and prepared by removing the seed coat. Then the seeds were soaked in 0.5% of 2, 3, 5 – Triphenyltetrazolium chloride solution and kept in the dark at 40^0C for 4 hours for staining. After staining, the seeds were soaked in 10 ml of2-methaxy ethanol (Methyl cellosolve) solution for 4 hours with occasional stirring till the extraction of red colourformazon was completed. The extract was decanted and the intensity of color was read in a spectrophotometer (ELICO SL 159) at 470 nm. The OD values were reported as dehydrogenase activity (Kittock and law, 1968).

Field Emergence (FE) Test

Four replicates of hundred seeds in each seed lot were sown in raised nursery beds and the seedlings emerged with normal root and shoot were counted after 15 days replication wise and the mean values were expressed in percentage.

$$\text{Field emergence (\%)} = \frac{\text{No. of normal seedlings}}{\text{Total no. of seeds sown}} \times 100$$

Statistical Analysis

Data obtained from the experiments were analyzed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at the 0.05 probability level. Values in percent data were arcsine transformed before analysis. Significance of correlation coefficients was tested by Pearson correlation method using SPSS software.

RESULTS AND DISCUSSIONS

Evaluation of Physiological Seed Quality Parameters

In the present study, the radicle emergence test and other seed vigor parameters for ten different seed lots were evaluated. Statistically significant differences were observed in all the physiological parameters among seed lots. Among the seed lots, L_1 recorded the highest values for all the observed parameters, *viz.*, speed of germination, germination per cent, root length, shoot length, dry matter production and vigor index(5.6, 88%, 12.4cm, 18.8cm, 0.557g / 10 seedling and 2746) respectively followed by L_3 (5.5, 87%, 12.3cm, 18.8cm, 0.549g / 10 seedling and 2706) respectively. While L_8recorded lowest values (4.2, 61%, 8.0cm, 16.1cm, 0.455g / 10 seedling and 1470) and L_9 (4.5, 63%, 8.4cm, 17.0cm, 0.451g / 10 seedling and 1600) respectively(Table 1).

The Mean Just Germination Time (MJGT) and Mean Germination Time (MGT) significantly increased with medium and low vigor seed lots compared with high vigor seed lots. The MJGT was minimum in L_1 and L_3(18.24 h) with short MJGT and the maximum in L_8 (20.88 h) followed by L_9 (20.40 h). However, the minimum MGT was registered in L_1 and L_3 (22.08) and the maximum MGT was L_8 (24.96) followed by L_9 (24.48 h) compared with other seed lots (Table 2). The current findings are in accordance with the results of radicle emergence studies in brinjal seed lots by Ozden*et al.* (2018).

Significant variations were observed in percentage radicle emergence (1mm and 2mm radicle length) among seed lots. The maximum radicle emergence per cent with 1mm length was observed within the prescribed time of 22 h in L_1 and L_3(88 %), while it was minimum in L_8(68 %). Further, an increase was noticed and 90 % and 89 % of seeds reached 2mm length of radicle with in next two hours (24 h) in seed lot L_1 and L_3, respectively. Whereas, in only $L_{8,}$62 % reached 2mm radicle length within the prescribed time of 24 hours. A delay in attaining 2mm radicle length was noticed in L_8 compared with other seed lots (Table 2).

Low vigor seed lots required more time to reach1mm and 2mm length of radicle emergence when compared to high vigor seed lots. Reasons for this delay attributed to has been interpreted as variations in the metabolic activity between the seeds with different vigor levels. The seed lots with high metabolic activity response quickly for imbibition and

proceed further for DNA repair, enzyme synthesis for breaking down of the food materials. while low vigor seeds respond slowly (Matthews and Powell, 2012). These results are in agreement with the findings of Mavi*et al.*(2010)in cucurbits and sweet corn seeds. Radicle emergence test is a good indicator for predicting field emergence potential, determining seed quality and classifying seed lots into different vigor status was also confirmed in pepper, cabbage, soybean and radish seeds (Demeir *et al.*, 2008; Matthews *et al.*, 2012).

Among ten different seed lots, significant differences were observed in field emergence percentage as well. Among the seed lots, L_1 and L_3 registered the highest field emergence (84%) and L_8 registered lowest field emergence of 53%(Table 2). The current findings are in accordance with the results of Ilbi and Eser (2006) in onion seed lots.

Evaluation of Biochemical Seed Quality Parameters

Statistically significant differences were observed for electrical conductivity (EC) of seed leachate (dsm^{-1}) in among seed lots. The seed lotL_1 registered the lowest EC value (0.562dsm^{-1})which was on par withL_3 (0.568dsm^{-1}), L_4 (0.580dsm^{-1}), L_2 (0.587dsm^{-1}). While L_8 registered the highest value(0.634dsm^{-1}) of electrical conductivity of seed leachate, which was on par with L_9 (0.622dsm^{-1}) (Fig. 1).

Similar results were also reported in groundnut seeds by Suganthi and Selvaraju(2017). The electrical conductivity of seed leachate was mainly governed by cell wall permeability. Higher EC indicated higher permeability, respiration rate and metabolic activity (Doijode, 1985). The increase in electrical conductivity might be due to the loss of selective permeability of cell membrane by autooxidation of polyunsaturated fatty acids, free radical peroxidation via auto-oxidation, lipo-oxygenase and hydrolytic damage (Francis and Coolbear, 1984)

Significant variations were also observed in dehydrogenase activity (OD value) among seed lots. The highest dehydrogenase activity was registered in L_1 (2.608), whileL_8 (2.418) registered the lowest value, which was on par with L_9 (2.421) and L_{10}(2.428) (Fig. 1).

Similar results were also reported in groundnut seeds. The activity of the dehydrogenase enzyme which is responsible for the respiration of the seed reduced with the ageing of the seeds, which is also used as a vigor indicator(Suganthi and Selvaraju, 2017).

Correlation between Seed Vigour Parameters and Radicle Emergence Test

Correlation analysis was carried out to assess the relationship between seed vigor parameters *viz.*, germination (%), mean just germination time (h), mean germination time (h), electrical conductivity of seed leachate (dsm^{-1}), dehydrogenase activity (OD Value), field emergence (%) with radicle emergence per cent with 1mm and 2mm radicle length in ten different seed lots. Among the various seed vigor parameters studied, field emergence (0.776**) followed by germination (0.719*) had a significant positive correlation with radicle emergence per cent with 1mm radicle length. The electrical conductivity of seed leachate (-0.702*) followed by mean germination time (-0.635*) had a significant negative correlation with radicle emergence per cent with1mm radicle length(Table 3).

However, field emergence (0.904**) followed by germination (0.892**) and dehydrogenase activity (0.757*) had a significant positive correlation with radicle emergence per cent with 2mm radicle length. Mean germination time (-0.829**) followed by Electrical conductivity of seed leachate (-0.812**) and mean just germination time (-0.807**) had a highly significant negative correlation with radicle emergence per cent with 2mm radicle length. The highest correlation

coefficient value was observed in 2mm radicle emergence percentage followed by 1mm radicle emergence percentage for most of the seed vigor parameters in ten different seed lots (Table 3).

Significant correlations between the mean germination time of 31 seed lots of maize and both the cold test and field emergence were also observed by Lovato *et al.*(2005). In coriander also the field emergence showed positive and significant correlation with radicle emergence test, accelerating ageing test and dehydrogenase activity test, whereas, it was negatively and significantly correlated with electrical conductivity of seed leachate in coriander (Kumar *et al.*, 2015)

Relationship between Seed Vigour Parameters and Radicle Emergence Test

Relationship between seed vigor parameters and radicle emergence percentage with 1mm and 2mm radicle length were analyzed. For most of the seed vigor parameters the highest R^2 value was observed in 2mm radicle emergence percentage (R^2 values: MJGT = 0.8839, MGT = 0.9223, EC = 0.8924, DA = 0.6335, GER = 0.9542 and FE = 0.9673)when compared to 1mm radicle emergence percentage (R^2 Values: MJGT = 0.8812, MGT = 0.9156, EC = 0.8330, DA = 0.6204, GER = 0.9525 and FE = 0.9504) in ten different seed lots (Fig. 2 & Fig. 3).

The current findings are in accordance with the results of Matthews and Powell(2011) in maize and Mavi *et al.* (2010) in cucumber, radish, and cotton

Navratil and Burris (1980) reported that the most predictive equations included both standard germination and time to 50% emergence in maize. Furthermore, the final emergence of the seed lots over five sowings seemed to be largely determined by the time taken to emerge, which was greatly influenced by temperature but was also significantly different among the lots. Similarly, three years of studies of four lots of hybrid corn by TeKrony *et al.*(1989) showed that low vigor lots emerge slowly and resulted in low field emergence.

CONCLUSIONS

Radicle emergence test is a quick test to predict different vigor level, field emergence potential and ranking seed lots. The radicle emergence test (2mm radicle length) was highly negatively correlated with mean germination time followed by the electrical conductivity of seed leachate and mean just germination time and it was positively correlated with field emergence followed by germination and dehydrogenase activity. The R^2 values between seed vigor parameters and radicle emergence test were significantly higher in 2mm length of radicle emergence when compared with the 1mm length of radicle emergence. Finally, the study concluded that 24 hour MGT with the attainment of 2mm radicle emergence percentage could be used as a quick method to assess the quality of okra seed lots by the seed analysts and seed industry.

AUTHORS CONTRIBUTIONS

Chinnasamy, G.P carried out, generated, analyzed the experiment interpreted the data and written the original draft. Dr. S. Sundareswaran, Dr. P. R. Renganayaki, and Dr. S. Srinivas an designed the experiment and corrected the manuscript as an advisory committee.

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APPENDICES

Table 1: Evaluation of Physiological Seed Quality Parameters in Different Seed Lots of okra var. Raja

Q.P / Seed Lots	Speed of Germination	Germination (%)	Root Length (cm)	Shoot Length (cm)	Dry Matter Production (g / 10 seedling)	Vigour Index
L_1	5.6	88 (69.73)	12.4	18.8	0.557	2746
L_2	5.2	83 (65.65)	11.4	17.0	0.475	2357
L_3	5.5	87 (68.86)	12.3	18.8	0.549	2706
L_4	5.3	86 (68.02)	11.3	17.1	0.517	2442
L_5	5.2	79 (62.72)	10.5	17.9	0.460	2244
L_6	5.0	71 (57.41)	10.4	16.6	0.457	1917
L_7	5.2	76 (60.66)	10.5	17.4	0.468	2120
L_8	4.2	61 (51.35)	8.0	16.1	0.455	1470
L_9	4.5	63 (52.53)	8.4	17.0	0.451	1600
L_{10}	4.7	64 (53.13)	9.1	17.0	0.433	1670
Mean	**5.0**	**76 (60.66)**	**10.4**	**17.4**	**0.482**	**2127**
SEd	0.08	1.04	0.12	0.20	0.0057	26.14
CD (P=0.05)	0.16	2.14	0.24	0.42	0.0116	53.38

(Figure in parenthesis indicate arcsine values) *Q.P - Quality parameters

Table 2: Comparison of Physiological Seed Quality Parameters in Different Seed Lots of okra var. Raja

Q.P / Seed lots	MJGT (hours)	MGT (hours)	Radicle emergence with 1mm length (%)	Radicle emergence with 2mm length (%)	Field emergence (%)
L_1	18.24	22.08	88 (69.73)	90 (71.56)	84 (66.42)
L_2	18.96	22.56	84 (66.42)	84 (66.42)	80 (63.43)
L_3	18.24	22.08	88 (69.73)	89 (70.63)	84 (66.42)
L_4	18.72	22.32	85 (67.21)	87 (68.86)	81 (64.15)
L_5	19.44	23.04	83 (65.65)	79 (62.72)	77 (61.34)
L_6	20.16	24.00	79 (62.72)	78 (62.02)	70 (56.79)
L_7	19.92	23.76	81 (64.15)	81 (64.15)	72 (58.05)
L_8	20.88	24.96	68 (55.55)	62 (51.94)	53 (46.72)
L_9	20.40	24.48	70 (56.79)	67 (54.94)	57 (49.02)
L_{10}	20.40	24.24	73 (58.69)	69 (56.16)	59 (50.18)
Mean	**19.54**	**23.35**	**80 (63.43)**	**79 (62.72)**	**72 (58.05)**
SEd	0.220	0.338	0.94	1.05	0.86
CD (P=0.05)	0.450	0.690	1.92	2.16	1.76
MJGT- Mean Just Germination Time (h); MGT- Mean Germination Time (h)					

(Figure in parenthesis indicate arcsine values)*Q.P - Quality parameters

Table 3: Correlation between Seed Vigour Parameters and Radicle Emergence Test in Different Seed Lots of okra var. Raja

	GER (%)	MJGT (h)	MGT (h)	EC (dsm^{-1})	DA (OD value)	FE (%)	RE 1 mm (%)	RE 2 mm (%)
GER (%)	1							
MJGT (h)	-.969**	1						
MGT (h)	-.982**	.991**	1					
EC (dsm^{-1})	-.883**	.894**	.885**	1				
DA (OD value)	.811**	-.865**	-.822**	-.827**	1			
FE (%)	.989**	-.946**	-.967**	-.892**	.775**	1		
RE 1 mm (%)	.719*	-.574	-.635*	-.702*	.541	.776**	1	
RE 2 mm (%)	.892**	-.807**	-.829**	-.812**	.757*	.904**	.885**	1
GER - Germination test				EC - Electrical conductivity of seed leachate test				

MJGT- Mean Just Germination Time	FE - Field emergence test
MGT- Mean germination time	RE 1 mm - Radicle emergence test (% of 1 mm radicle length)
DA- Dehydrogenase activity test	RE 2 mm - Radicle emergence test (% of 2 mm radicle length)
****. Correlation is significant at the 0.01 level**	***. Correlation is significant at the 0.05 level**

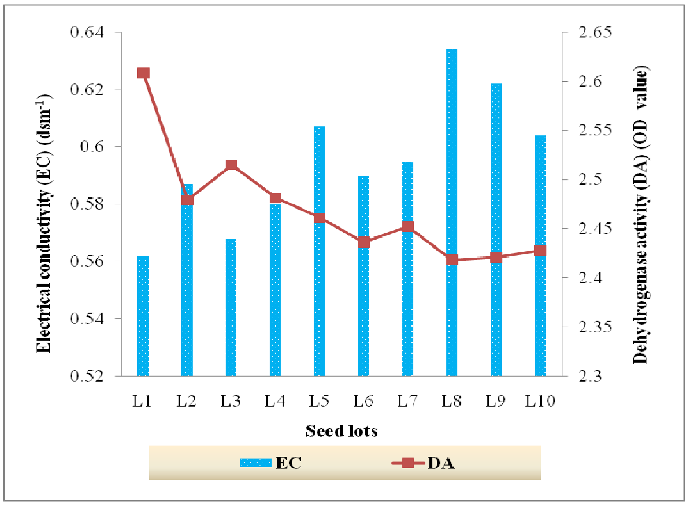
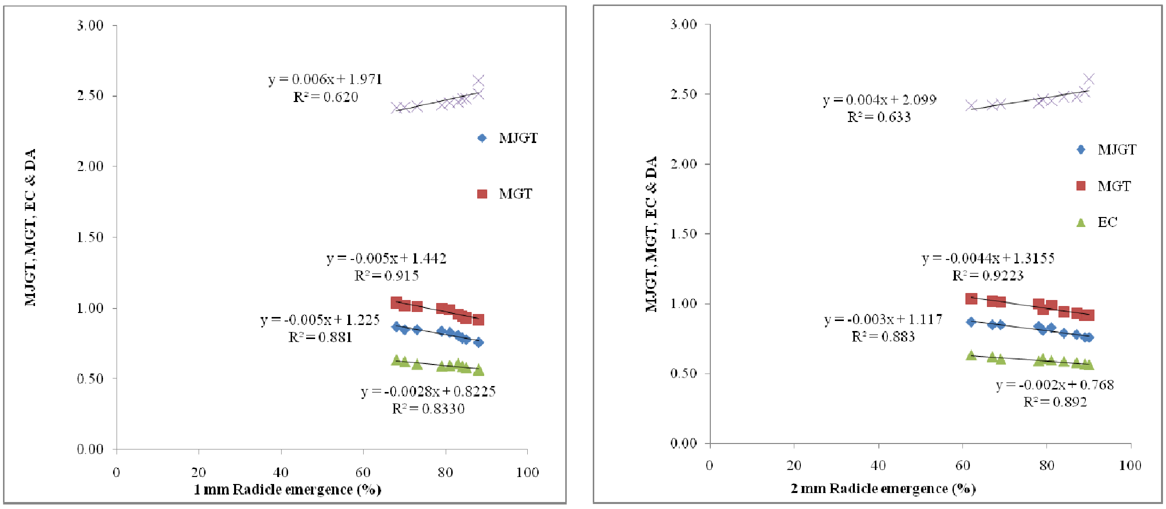
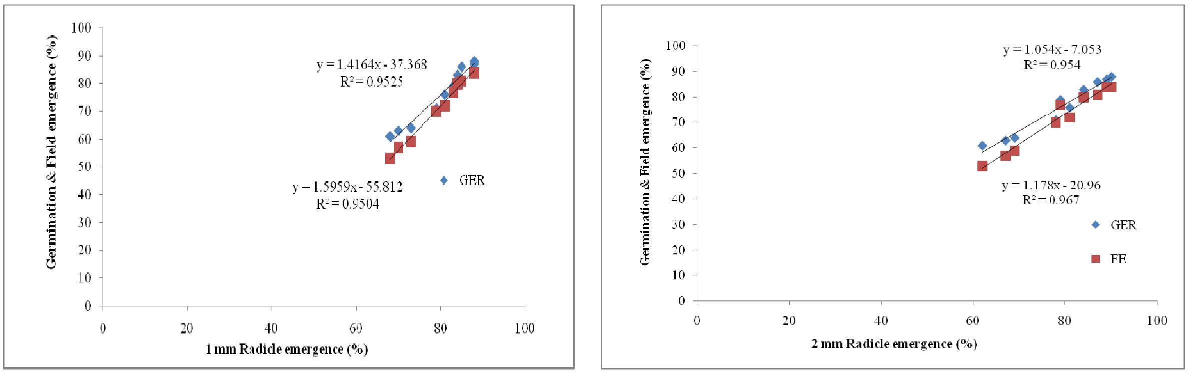


Figure 1: Electrical Conductivity of Seed Leachate (Dsm^{-1}) and Dehydrogenase Activity (OD Value) In Different Seed Lots of Okra Var. Raja



MJGT- Mean just germination time (h)	EC - Electrical conductivity of seed leachate test (dsm^{-1})
MGT- Mean germination time (h)	DA- Dehydrogenase activity test (OD value)

Figure 2: Relationship Between Seed Vigour Parameters and Radicle Emergence Percentage (1 Mm & 2 Mm Length) in Different Seed Lots of Okra Var. Raja



GER - Germination (%) FE - Field emergence (%)

Figure 3: Relationship Between Germination (%), Field Emergence (%) and Radicle Emergence (%) (1 mm & 2 mm Length) in Different Seed Lots of Okra Var. Raja